Effect of HIV Infection and Antiretroviral Therapy on Lipid Profile of HIV Subjects in Warri, Delta State, Nigeria

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ABSTRACT

This work was designed to study the impact of HIV infection and anti-retroviral therapy on the lipid profile of HIV patients attending Voluntary Counselling and testing centre at Government Hospital and Sage Clinic, Warri. Two hundred and fifty three subjects comprising; 85 HIV patients on ART, 90 HIV patients not on ART and 78 age-matched apparently healthy HIV negative controls were recruited for the study. Subjects were of both sexes within the age range of 18-60 years. Informed consent was obtained from all participants. Ethical approval was obtained from the ethics committee of Delta State Hospital Management board. Total cholesterol, high density lipoprotein-cholesterol and triglyceride were evaluated using routine enzymatic colorimetric kits while low and very low density lipoprotein were extrapolated using Friedewald’s formula. SPSS version 21 was used for statistical data analysis. Total cholesterol (T-Chol), triglyceride (TG), low density lipoprotein (LDL)-cholesterol, very low density lipoprotein (VLDL)-cholesterol were significantly higher in HIV-positive subjects on ART and those not on ART compared to the controls (p<0.05). No significant difference was observed in the high density lipoprotein (HDL)-cholesterol in these groups (p>0.05). VLDL-C and TG was significantly higher in female control subjects than the male controls (P<0.05), while there was no significant gender difference in all the parameters in HIV positive on ART and those not on ART. HIV and ART affects lipid metabolism of HIV-positive patients as inappropriate increased serum cholesterol and triglyceride could be as a result of HIV infection which is aggravated with ART. However, it seems that neither HIV nor ART affects the level of high density lipoprotein-cholesterol. Therefore, HIV-positive subject should be assessed periodically for lipid profile before enrolment for HAART and during therapy given the high incidence and risk of cardiovascular disease.

KEYWORDS: Antiretroviral therapy, Cardiovascular diseases, Cholesterol, and HIV.

INTRODUCTION

Since its identification in 1981, human immunodeficiency virus (HIV) infection and associated acquired immune deficiency syndrome (AIDS) remain a major burden globally[1]. Nigeria is the most populous black nation in the world with a population of 160 million. It has the second highest number of people living with HIV (3.1 million) next to South Africa (5.6 million). It accounts for 10% of the global HIV burden. Approximately, two hundred and fifteen
Cardiac risk factors are known to exist in both HIV-positive and HIV-negative individuals. These risk factors range from hypercholesterolaemia and hypertriglyceridaemia to a family of heart disease. It has been found that HIV-positive patients possess higher titre of circulating adhesion molecules than normal subjects and a seven percent absolute risk of developing heart disease within a decade. In addition, some indirect evidence from retrospective cohort analysis and non-invasive imaging of peripheral arteries indicate that HIV-positive individuals are at higher risk for arteriosclerosis than HIV-negative individual [3]. Metabolic abnormalities such as dyslipidaemia among HIV infected patients result in significant morbidity including increased cardiovascular disease (CVD) risk [4]. HIV buds from lipid rafts and requires cholesterol for its egress from and entry into cell. Viral accessory protein Nef plays a major role in this process. It not only increases the biosynthesis of lipid raft and viral particles with newly synthesized cholesterol, but also enriches them[5].

The introduction of anti-retroviral therapies has led to a remarkable increase in the life expectancy of patients with HIV infection. Unfortunately, current treatment may cause a wide spectrum of metabolic disturbance and comorbid conditions, with cardiovascular disease as an important example. HIV seems to raise serum lipid levels as do the anti-retroviral drugs used to treat it. Dyslipidaemia is particularly frequent and is mostly characterised by increased triglyceride and low HDL-Cholesterol. HIV has been associated with dyslipidaemia independent of antiretroviral therapy (ART). ART can also contribute to dyslipidaemia. Dyslipidaemia has been described as being more common and more severe in HIV patients receiving ART than in patients not on therapy. The severity of the dyslipidaemia and the typical pattern of the lipid profile differ among and within the classes of ART. Dyslipidaemia is also observed in treatment-naïve HIV-infected patients, suggesting that HIV infection itself has a metabolically deleterious effect [6].

Also dyslipidaemia does not develop in everyone who takes these medications, suggesting that the host factors play a major role in its development [7]. Non-nucleoside reverse transcriptase inhibitors have been associated with elevated level of high density lipoprotein-cholesterol and total cholesterol. Nucleoside reverse transcriptase inhibitors on the other hand, are heterogeneous in their lipid effects, which may depend somewhat on interactions with other anti-retroviral drug regime. Protease inhibitors are generally associated with elevated levels of total cholesterol and triglycerides [7]. The interplay between HIV and cholesterol is complex. HIV appears to increase cholesterol synthesis and uptake in productively infected cells to ensure HIV infectivity [8]. Additionally, many HIV patients have low levels of HDL-cholesterol and these levels are further reduced by ART. The effects of highly active anti-retroviral therapy on the pathogenesis of dyslipidaemia are compounded by environmental, genetic, nutritional and behavioural factors [9]. Increased high density lipoprotein cholesterol (HDL-C) is associated with improved prognosis in coronary heart disease and may exert a protective role [10].

HIV infected individuals have metabolic abnormalities that put them at increased risk of cardiovascular disease [CVD], including abnormalities associated with HIV infections itself, antiretroviral treatment, restoration to health, and body composition changes. These facts have been extensively researched in the developed world. However with the obvious paucity of research data on these parameters in Nigeria and the variation in the distribution of HIV strains in different locations, this research was designed to assess the lipid profile of HIV patients.

MATERIALS AND METHODS

Subject selection
A total of Two hundred and fifty three (253) subjects (90 males and 163 females) between the ages of 16-58yrs were recruited for this study from the Voluntary Counselling and Testing (VCT) centre in Delta State General Hospital and Sage Clinic both in Warri, Delta State. The subjects were grouped as follows;

- Group I: HIV positive subjects on Anti-retroviral drugs (HIV/ART).
- Group II: HIV positive not on anti-retroviral therapy (HIV).
- Group III: Apparently healthy age-matched HIV-seronegative participants (Controls)

Group J1: Females on ART (HIV/ART)
Group K1: Males on ART (HIV/ART)
Group J2: Female not on ART (HIV)
Group K2: Male not on ART (HIV)
Group J3: Female controls
Group K3: Male controls

Ethical approval was obtained from the ethics committee of Delta State Hospital Management board and informed consent was obtained from all participants that were involved in this study.

Inclusion/Exclusion criteria
Inclusion criteria were HIV positive patients on antiretroviral therapy (ART) at the duration of three months to thirty-eight months, HIV positive patient not on ART and HIV negative patients as control subjects. Exclusion criteria were pregnant women, known bleeding or clotting disorders, including history of deep vein thrombosis. Concurrent malignancy requiring cytotoxic chemotherapy or radiation therapy. And subjects that declined from participating in the study.

Sample collection.
Four (4) millilitres of fasting blood sample was collected from the ante-cubital fossa of each subject into plain containers (chemically clean plastic tube), which was allowed to clot and the serum used for the HIV screening and estimation of Lipid profile.

METHODS
HIV 1/2 screening was carried out by rapid test kit by immunochromatography using
1. Determine HIV ½ test strips (Alere medical company Ltd, Japan, 2013, lot No: 53427K100)
2. STAT PAK HIV ½ test kit (CHEMBIO DIAGNOSTIC SYSTEMS INC., USA 2012, lot No: HIV070612)
3. UNI-GOLD HIV ½ test kit (TRINITY BIOTECH PLC, Ireland 2013, lot HIV3100204) as tie- breaker

**Lipid profile estimation**

a) High density lipoproteins-cholesterol (HDL-C) using analytical grade enzymatic colorimetric kits from Randox laboratory Ltd.

b) Total Cholesterol (T-CHOL) using analytical grade enzymatic colorimetric kits from Randox Laboratory LTD.

c) Triglyceride (TG) using qualitative method by TECO DIAGNOSTICS LTD.

d) Low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were calculated by Friedewald’s formula as follows;

\[ \text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C}) \]

\[ \text{VLDL-C} = \frac{\text{TG}}{5} \]

**Statistical analysis**

The results were statistically analysed using the Statistical Package for Social Sciences (SPSS) version 21. Data were expressed as mean ± SD. Analysis of variance (ANOVA) was used to compare differences among groups, while student t-test was used to compare the differences between groups. Values were considered significant at P<0.05.

**RESULTS**

There was a significantly higher mean value of serum total cholesterol (mg/dl) in HIV-positive subjects (ART and non-ART) compared with control subjects (p<0.05). Similarly, mean ± S.D serum triglyceride (mg/dl) in HIV-positive subjects (ART and non-ART) was significantly higher than control subjects (p<0.05) in each case. The mean ± S.D serum low density lipoprotein-cholesterol (mg/dl) was significantly higher in HIV-positive subjects (ART and non-ART) compared with control subjects (p<0.05).

Similarly, mean value of very low density lipoprotein-cholesterol (mg/dl) in HIV-positive subjects (ART and non-ART in each case) was significantly higher compared with corresponding control subjects (p<0.01). Also, significantly higher value was observed in the ratio of total cholesterol and high density lipoprotein-cholesterol in HIV-positive subjects (ART and non-ART) in each case when compared with control subjects (p<0.05). The mean ± S.D of serum triglyceride and very low density lipoprotein-cholesterol was significantly higher in HIV-positive on ART compared with the corresponding values in HIV-positive not on ART (p<0.05). See Table 1

The mean values of TG and VLDL-C was significantly higher in females compared to male control subjects (P<0.05). See Table 2.

<table>
<thead>
<tr>
<th>T-Chol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>T-Chol/ HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/ART (N=85) (I)</td>
<td>191.33±28.61</td>
<td>49.28±9.68</td>
<td>125.91±36.24</td>
<td>116.56±24.76</td>
<td>25.06±7.26</td>
</tr>
<tr>
<td>HIV (N=90) (II)</td>
<td>190.00±36.64</td>
<td>48.90±15.00</td>
<td>111.12±33.56</td>
<td>118.52±25.79</td>
<td>22.31±6.69</td>
</tr>
<tr>
<td>Control (N=78) (III)</td>
<td>169.60±25.41</td>
<td>50.77±9.54</td>
<td>90.53±26.57</td>
<td>99.86±20.34</td>
<td>18.11±5.33</td>
</tr>
<tr>
<td>F (P Value)</td>
<td>12.589(0.000*)</td>
<td>0.571(0.566)</td>
<td>24.151(0.000*)</td>
<td>14.995(0.000*)</td>
<td>23.416(0.000*)</td>
</tr>
<tr>
<td>I vs. II (P Value)</td>
<td>0.961</td>
<td>0.978</td>
<td>0.016*</td>
<td>0.865</td>
<td>0.027*</td>
</tr>
<tr>
<td>I vs. III (P Value)</td>
<td>0.000*</td>
<td>0.586</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>II vs. III (P Value)</td>
<td>0.000*</td>
<td>0.593</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Key: F (P value): mean ± SD of Lipid profile (T-Chol. (mg/dl), HDL-C (mg/dl), TG (mg/dl), LDL-C (mg/dl), VLDL-C (mg/dl), ratio of T-Chol and HDL-C) compared among HIV-positive subjects on ART, HIV-positive subjects not on ART and control subjects using ANOVA.

I vs. II (P value): mean ± SD compared between HIV-positive subjects on ART and HIV-positive subjects not on ART using Student’s t-test.

I vs. III (P value): mean ± SD compared between HIV-positive subjects on ART and control subjects using Student’s t-test.

II vs. III (P value): mean ± SD compared between HIV-positive subjects not on ART and control subjects using Student’s t-test.
Table 2: Mean ± SD of lipid profile among HIV-positive female and male on ART, HIV-positive female and male not on ART and female and male control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>T-Chol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>T. Chol/HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/ART (Female) (N=55) (J₁)</td>
<td>186.71±24.98</td>
<td>49.80±9.66</td>
<td>131.87±33.90</td>
<td>110.97±17.28</td>
<td>26.19±6.83</td>
<td>3.84±0.57</td>
</tr>
<tr>
<td>HIV/ART (Male) (N=30) (K₁)</td>
<td>199.80±33.09</td>
<td>48.33±9.81</td>
<td>114.97±38.38</td>
<td>126.81±32.44</td>
<td>22.99±7.67</td>
<td>4.03±0.85</td>
</tr>
<tr>
<td>P Value</td>
<td>0.419</td>
<td>0.985</td>
<td>0.345</td>
<td>0.153</td>
<td>0.406</td>
<td>0.878</td>
</tr>
<tr>
<td>HIV(Female) (N=60) (J₂)</td>
<td>191.15±37.37</td>
<td>49.27±11.93</td>
<td>114.95±29.86</td>
<td>119.58±26.32</td>
<td>22.99±5.79</td>
<td>3.96±0.53</td>
</tr>
<tr>
<td>HIV (Male) (N=30) (K₂)</td>
<td>187.70±35.66</td>
<td>48.17±19.99</td>
<td>103.47±40.74</td>
<td>116.42±24.99</td>
<td>20.95±8.14</td>
<td>4.21±0.65</td>
</tr>
<tr>
<td>P Value</td>
<td>0.998</td>
<td>1.000</td>
<td>0.739</td>
<td>0.993</td>
<td>0.822</td>
<td>0.465</td>
</tr>
<tr>
<td>Control (Female) (N=48) (J₃)</td>
<td>170.02±18.68</td>
<td>51.83±7.20</td>
<td>106.73±19.39</td>
<td>96.79±14.96</td>
<td>21.38±3.85</td>
<td>3.31±0.40</td>
</tr>
<tr>
<td>Control (Male) (N=30) (K₃)</td>
<td>168.93±33.89</td>
<td>49.07±12.36</td>
<td>64.60±11.61</td>
<td>104.77±26.37</td>
<td>12.89±2.35</td>
<td>3.46±0.56</td>
</tr>
<tr>
<td>P Value</td>
<td>1.000</td>
<td>0.873</td>
<td>0.000*</td>
<td>0.658</td>
<td>0.000*</td>
<td>0.770</td>
</tr>
</tbody>
</table>

Key: F (P value): mean ± SD of Lipid Profile (T-Chol (mg/dl), HDL-C (mg/dl), TG (mg/dl), LDL-C (mg/dl), VLDL-C (mg/dl), ratio of T-Chol and HDL-C) compared among HIV-positive female and male subjects on ART, HIV-positive female and male subjects not on ART and control female and male subjects using ANOVA.

J₁ vs. K₁ (P value): mean ± SD compared between HIV-positive female and male subjects on ART using Student’s t-test.

J₂ vs. K₂ (P value): mean ± SD compared between HIV-positive female and male subjects not on ART using Student’s t-test.

J₃ vs. K₃ (P value): mean ± SD compared between control female and male subjects using Student’s t-test

DISCUSSION

The interplay between HIV and cholesterol is complex. HIV appears to increase cholesterol synthesis and uptake in productively infected to ensure HIV infectivity. Previous data suggest that individual with high level cholesterol have high viral rebound [8]. This study observed increase in serum total cholesterol concentration, serum triglyceride, low density lipoprotein-cholesterol, very low density lipoprotein-cholesterol and the ratio of total cholesterol to high density lipoprotein-cholesterol in HIV-infected subjects both on ART and those not on ART compared with the control subjects. These observed increase in some lipid profile parameters could be explained in part by the increase in oxidative stress and lipid peroxidation associated with HIV/AIDS [11] and also could be from the high level of pro-inflammatory cytokines (Tissue Necrotic Factor-alpha, Interleukin-6, Interleukin-1) observed in these patients with an active infection, due to their increased secretion by activated monocytes and macrophages [11].

Tissue Necrotic Factor-alpha, Interleukin-6 can decrease the expression and activities of lipoprotein lipase, which is involved in triglyceride clearance from circulation lipoproteins. Very low density lipoproteins are composed predominantly of triglycerides. This may explain why very low density lipoprotein is also elevated when the levels of triglycerides is increased among the HIV-positive subjects. Thus, the observed increase in serum triglyceride level could be due to increase in very low density lipoprotein-cholesterol and also to triglyceride-enriched low density lipoprotein [12]. However, both decreased triglyceride clearance and increased very low density lipoprotein-cholesterol over-production have been found in HIV-positive patients [13] and this could also explain the increased serum triglyceride observed.

However, no difference was observed in serum high density lipoprotein between these groups. This could be explained by an earlier finding that acute-phase proteins can bind to high density lipoprotein cholesterol particles promoting their uptake by macrophages and therefore increases their clearance rate [3]. This agrees with previous findings [11] that reported no significant difference in the mean serum high density lipoprotein-cholesterol level of HIV-positive subjects on ART and those not on ART compared with control subjects.

The result of this study shows that the total cholesterol levels were not significantly different in the males compared to females on ART and those not on ART. For those on ART, this could be as a result of the short duration of the commencement of ART in the subjects recruited in this study as there have been reported cases of disturbances in cholesterol metabolism upon ART treatment in a previous study [14]. This finding however disagrees with earlier studies that reported a significant increase in serum total cholesterol levels of the male and female HIV-positive subjects on ART and those without ART compared to...
control subjects which they attributed to more predisposition to hypercholesterolemia due to the HIV-infection and direct effect of the drugs, particularly in the liver, resulting in modified lipid metabolism.

CONCLUSION

HIV and ART have effects on lipid metabolism of HIV-positive patients. ART was associated with a significant increase in Triglyceride and Very Low Density Lipoprotein-cholesterol. There was no sex difference observed in their pattern of lipid profile. It seems that neither HIV nor ART could affect the level of high density lipoprotein-cholesterol. Inappropriate increased serum cholesterol and triglyceride may be as a result of HIV infection which could be aggravated with ART.

RECOMMENDATION

HIV-positive subject should be assessed periodically for lipid profile before enrolment for HAART and during therapy given the high incidence and risk of cardiovascular disease. Viral load assay should be undertaken with a larger sample size to ascertain the possible effect of viraemia on these parameters.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exists in this research work.

REFERENCES


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